Fibrinogen concentrate in dilutional coagulopathy: a dose study in pigs

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Dilutional coagulopathy can be reversed with fibrinogen concentrate. Effects of different fibrinogen dosages on clot function are not defined; high doses may increase the risk of thromboemboembolism. This study investigated the effect of six fibrinogen dosages on coagulation profile and blood loss in coagulopathic pigs. Forty-two pigs underwent a 60% hemodilution (HD) with hydroxyethylstarch (HES 130/0.4). After a standardized bone injury, animals randomly received 37.5, 75, 150, 300, 450 or 600 mg/kg fibrinogen (FGTW, LFB) or 500 ml of saline. Four hours later, a standardized liver injury was performed. Animals were then observed for two hours or until death. Blood loss was measured after death; Hemodynamic and coagulation parameters (thromboelastometry) were measured at baseline (BL), after HD, 15', 1, 2, 4 hours after fibrinogen administration and 2 hours after liver injury or right before the animals' death. Occurrence of thrombosis was examined in histological slides of internal organs. Statistical significance was set at $p < 0.05$.

Doses of 150 mg/kg fibrinogen and higher reversed dilutional coagulopathy: Maximum clot firmness (MCF) was decreased after hemodilution (36 ± 3 mm vs. 65 ± 4 mm at BL, $p < 0.05$) and returned to BL after fibrinogen administration (69 ± 5 mm). Blood loss was significantly decreased with increased fibrinogen dosages: 42 ± 19 (sham), 34 ± 14 (75 mg/kg), 29 ± 13 (150 mg/kg), 28 ± 10 ml/kgbw (600 mg/kg). Fibrinogen (150-600 mg/kg) normalized clot firmness and decreased blood loss. No signs of hypercoagulability or thromboembolism were detected after high dosages.

In massive bleeding, fibrinogen reaches critical levels at an early stage, generally before any other procoagulant coagulation factor, including platelets (PLTs), as a consequence of dilution and consumption.[1-4] Even relatively small quantities of colloids (<1000 mL) impair fibrin polymerization primarily.[5,6] McLoughlin and colleagues[5] showed that in normovolemic dilution with a starting fibrinogen level of less than 300 mg/dL, the critical fibrinogen concentration of 100 mg/dL is reached before a critical hematocrit (Hct) value would necessitate administration of red blood cells (RBCs). Clinical data from gynecology,[7] neurosurgery,[8] and cardiac surgery[9,10] show that the peri- and postoperative bleeding tendency is increased when fibrinogen levels are below 150 to 200 mg/dL. Singbartl and colleagues[11] developed a mathematical model, which underlines the importance of particularly paying attention to the patients' fibrinogen level instead of only focusing on Hct values as a potential transfusion trigger.

In vitro studies and experimental investigations,[12-16] as well as reports from postmarketing surveillance and retrospective data analyses,[17,18] have consistently shown

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that fibrinogen can increase clot firmness. In a recent prospective randomized placebo-controlled clinical trial, Fenger-Eriksen and colleagues[19] showed that in patients undergoing radical cystectomy postoperative transfusions can be reduced if intraoperatively occurring coagulopathy is corrected with fibrinogen administration. Stinger and colleagues[20] showed that in severely injured soldiers admitted to a combat hospital, survival was improved with increased administration of fibrinogen (in the form of cryoprecipitate and fresh-frozen plasma).

Despite these positive results obtained by administration of fibrinogen, there is still uncertainty regarding the upper limit of fibrinogen concentrate that can be infused under circumstances of dilutional coagulopathy. In view of different studies proposing chronically elevated plasma fibrinogen levels to be of major relevance for cardiac events, especially in diabetic patients,[21-24] the current literature is lacking data regarding the upper limit of fibrinogen concentrate that can be administered under circumstances of dilutional coagulopathy. The question whether high fibrinogen dosages represent a safety issue leading to an increased risk of thrombosis has not yet been addressed.

This study was conducted to investigate the effect of fibrinogen dose on coagulation and blood loss in a well-established pig model for dilutional coagulopathy, augmented with a standardized bone injury, followed by a central liver injury. Changes of coagulation function were assessed by thromboelastometry and measurements of blood loss as well as clot weight.

MATERIALS AND METHODS

The following study was approved by the Austrian Federal Animal Investigational Committee. Animal care and use were performed by qualified individuals and all the facilities and transportation comply with current legal requirements and guidelines. Anesthesia was used in all surgical interventions, and all unnecessary suffering was avoided and research was terminated if unnecessary pain or distress resulted.

Animal model

A total of 42 male and female pigs (German domestic pigs, 12-14 weeks old, 25-35 kg) were included in this study. Animals were fasted overnight with free access to water. Animals were medicated with azaperone (4 mg/kg intramuscularly, Stresnil, Janssen, Vienna, Austria) and atropine (0.1 mg/kg intramuscularly) 1 hour before the start of the experiment. Anesthesia was induced with propofol (1-2 mg/kg intravenously). Analgesia was provided by injection of piritramide (30 mg, Dipidolor, Janssen, Vienna, Austria). After being intubated during spontaneous respiration, animals were mechanically ventilated with a

volume-controlled ventilator (EVITA 2, Draeger, Lübeck, Germany) first with 100% (until the tracheal tube was safely secured) and then with 35% oxygen; tidal volume was adjusted to maintain normocapnia. Anesthesia was maintained with propofol (6-8 mg/kg/hr). Muscle relaxation was induced with rocuronium (0.6 mg/kg/hr; Esmeron, N.V. Organon, Oss, the Netherlands) after endotracheal intubation. A 6.0 Fr catheter was advanced into one femoral artery for invasive blood pressure measurement and both femoral veins as well as one subclavian vein were cannulated with 8.5 Fr catheters. Subsequently, a Swan-Ganz catheter was introduced via the subclavian vein. The basal need for fluid replacement (4 mL/kg body weight) was performed with crystalloids (Ringer's lactate, Fresenius Kabi, Graz, Austria). Analgesia was maintained by repeated injections of 15 to 30 mg of piritramide according to the animals' need, which was assessed throughout the entire experiment by continuous monitoring of blood pressure and heart rate. An acute increase in blood pressure and heart rate without performing any manipulations on the animal was considered a sign of distress, which was immediately followed by an injection of piritramide. Additionally, 5 to 10 minutes before an expected painful intervention, for example, bone injury or laparotomy and liver injury, an additional bolus of 30 mg of piritramide was administered. This anesthetic regimen has been shown to ensure a constant anesthetic plane providing sufficient anesthesia and analgesia during the entire experiment. All animals were comparable regarding their consumption of piritramide, which was between 90 and 100 mg for the entire time of the experiment.

Experimental protocol (Fig. 1)

After instrumentation and baseline (BL) measurements, animals underwent a 60% normovolemic hemodilution (HD) with 6% hydroxyethyl starch (HES) 130/0.4 (Voluven, Fresenius Co., Bad Homburg, Germany). Blood was withdrawn via the large catheters and replaced by colloid with a relationship of 1:1. For an estimated exchange of approximately 60% of total blood volume, animals with a weight of 30 kg were infused with 1.100 mL of 6% HES 130/0.4. The normovolemic HD was completed when the resulting coagulopathy had reached a maximum clot firmness (MCF) of less than 40 mm, measured by thromboelastometry. To prevent a hemodynamically relevant anemia, animals were retransfused the RBCs collected and concentrated by a cell saver system (Cats, Fresenius) to achieve a hemoglobin (Hb) value between 5 and 6 g/dL.

A standardized bone injury was then performed by drilling a 3-mm hole into the neck of the tibia at a depth as to penetrate the marrow. Five minutes after bone injury, plasma fibrinogen concentration was measured, followed by administration of human fibrinogen concentrate (FGTW, Laboratoire Français du Fractionnement et des Biotechnologies, LFB, Paris, France) over 30 minutes.
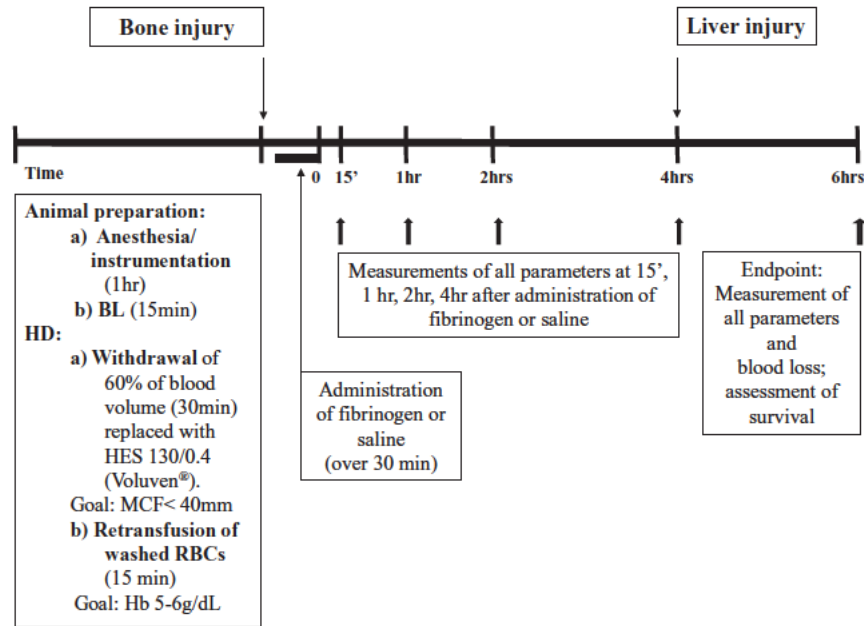
Fig. 1. Experimental protocol.

Excess blood was suctioned from the wound area between the bone and muscle and pooled in a collection jar. If blood loss from bone injury exceeded 500 mL, bleeding was stopped by compression with a standard gauze bandage to ensure that the pig was hemodynamically stable for the next 4 hours of observation. Fifteen minutes after the completion of fibrinogen administration, additional determinations of all measured variables were performed. Further on, 1, 2, and 4 hours after administration of fibrinogen, blood samples were taken for coagulation measurements. Four hours after administration of fibrinogen, a standardized liver injury was induced by making a central incision of the falciforme ligament over the central liver lobe using a template. The resulting liver incision was 8 cm long and 2 cm deep. Two hours after liver injury or immediately before death measurements of all variables were carried out. Surviving animals were euthanized via potassium infusion. Two hours after liver injury or right after euthanasia of the surviving animals, blood loss was assessed by suctioning the blood out of the intestinal cavity into a collection jar, which weight was known. Blood loss was then quantified by weighing the collection jar and subtracting the weight of the jar. After the intestinal cavity was completely free of blood, the clot, which had formed on the liver surface, right above the liver incision site, was removed into a jar and weighed.

Macro- and microscopic examination for thrombosis

At the end of each experiment tissue samples of lungs, heart, kidneys, and liver were dissected from every animal and macroscopically examined for the presence of thrombi. Additional tissue samples of these organs were immediately immersed into a 10% formalin solution. After dehydration through an ascending sequence of alcohol, samples were imbedded in paraffin and cut into 7-μm slides. Slides were then stained with the classic hematoxylin-eosin dyeing technique and microscopically scanned for the presence or absence of microvessel thrombi.

The investigators of the study involved in examining the histologic slides were blinded to group assignment; investigators who performed the macroscopic examination were not blinded.

Experimental groups

By using a computer program (DatInf RandList, DatInf GmbH, Tübingen, Germany) animals were randomized into seven groups receiving increasing fibrinogen dosages or saline, respectively (A, 37.5 mg/kg; B, 75 mg/kg; C, 150 mg/kg; D, 300 mg/kg; E, 450 mg/kg; F, 600 mg/kg; and G, saline group, 500 mL of saline). Each group included six pigs.

Blood sampling and analytical methods

Blood samples were drawn from the femoral artery. Samples for ROTEM and coagulation analysis were collected in standard sodium citrate tubes (Sarstedt, Nuermbrecht, Germany). INTEM and EXTEM tests were used in the study. By using the INTEM reagent (partial thromboplastin phospholipid and ellagic acid) coagulation is
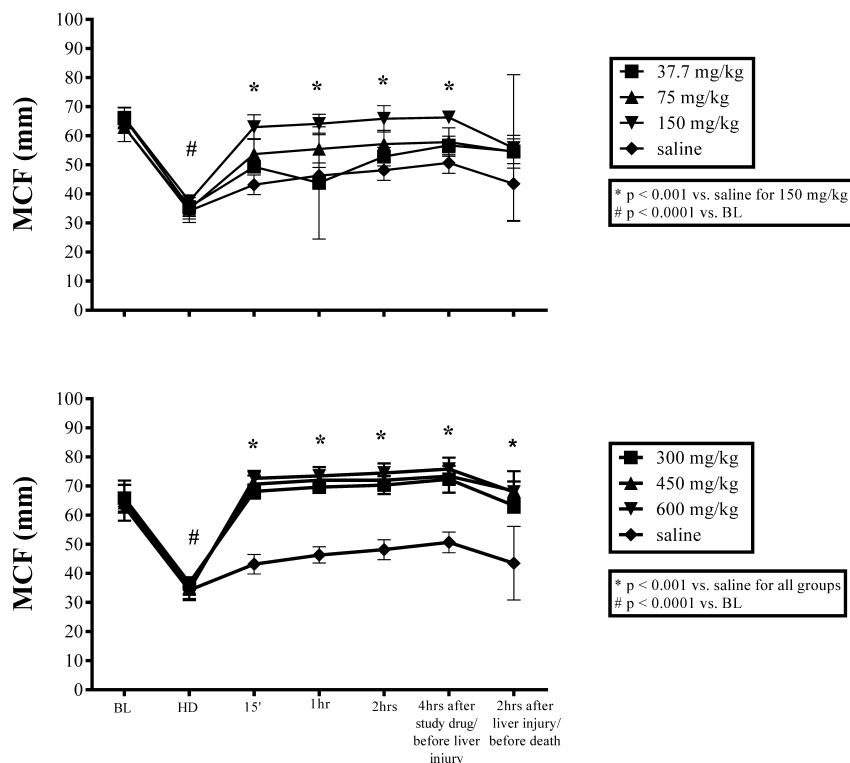
Fig. 2. INTEM MCF significantly decreased after HD with HES 130/0.4. Administration of fibrinogen increased MCF in a dose-dependent manner. (■) 37.7 mg/kg; (▲) 75 mg/kg; (▼) 150 mg/kg; (◆) saline. *p < 0.001 versus saline for 150 mg/kg; #p < 0.0001 versus BL.

activated though the intrinsic pathway, whereas the EXTEM reagent (recombinant tissue factor and phospholipids) activates coagulation via the extrinsic pathway. Previous studies have shown that FIBTEM (cytochalasin D) performed in swine blood cannot exclude PLT contribution since swine PLTs cannot be completely blocked by cytochalasin D.[13,25] Therefore, we performed EXTEM in plasma, which can functionally be interpreted as FIBTEM.

Blood samples for blood cell count were collected in standard EDTA tubes (Sarstedt). Prothrombin time (PT), activated partial thromboplastin time (aPTT-LA1), fibrinogen concentration, antithrombin, and thrombin-antithrombin were determined by standard laboratory methods using the appropriate tests (Dade Behring, Marburg, Germany; and the Amelung Coagulometer, Baxter, UK). For D-dimer measurements the assay D-dimer 0020008500 (Instrumentation Laboratory Company, Lexington, KY) was used. Blood cell count was performed using the cell counter (Sysmex Poch-100i counter, Lake Zurich, IL).

Statistical analysis

The Shapiro-Wilk's test was used to check normality distribution of study variables. Analysis of variance for repeated measurements followed by the Bonferroni post-

test was used to assess differences between study groups at different time points. Nonparametric testing was performed using Kruskal-Wallis test. A t test was used to compare specific variables at BL versus after HD.

A Jonckheere-Terpstra's test was used to assess whether the total blood loss correlates with fibrinogen dosage. Since the distribution of the total blood loss is nonnormal, this nonparametric test for ordered differences among treatment groups is appropriate. The Jonckheere-Terpstra's test tests the null hypothesis that the distribution of total blood loss does not differ among the fibrinogen dosage groups. All data are presented as mean ± standard deviation unless otherwise noted in the figure legend. A p value of less than 0.05 was considered significant.

RESULTS

Hemodynamic and blood gas variables

Hemodynamic and blood gas variables followed the same trend in all animals, and no significant differences were found between groups. From BL until liver injury no significant changes in hemodynamic variables occurred (BL mean arterial pressure [MAP], 68 ± 10 mmHg; 4 hr after fibrinogen administration/right before liver injury, 63 ± 10 mmHg); after liver injury MAP and central venous pressure significantly dropped whereas heart rate significantly increased compared to BL in all groups (BL MAP 68 ± 10 vs. 33 ± 21 mmHg after liver injury; BL central venous pressure 6 ± 1 vs. 3 ± 1 mmHg after liver injury; BL heart rate 76 ± 9 vs. 128 ± 56 bpm; p < 0.05). Serum lactate concentrations slightly decreased from BL until 4 hours after fibrinogen administration (BL, 23 ± 10 vs. 11 ± 4 mmol/L; changes not significant) and significantly increased in all groups after liver injury (48 ± 30 mmol/L; p < 0.05). There was no difference in lactate concentrations among groups.

Functional coagulation assessment

ROTEM variables were impaired after HD with 6% HES 130/0.4: INTEM clotting time increased to 184 ± 28 seconds versus 141 ± 24 seconds at BL and INTEM MCF (Fig. 2) decreased to 36 ± 3 mm versus 65 ± 4 mm at BL after infusion of the colloid (p < 0.0001 for both variables vs. BL). In the same way alpha angle decreased (BL, 79 ± 12°; after HD, 60 ± 7°) and clot formation time increased after HD (BL, 48 ± 9 sec; after HD, 191 ± 48 sec;
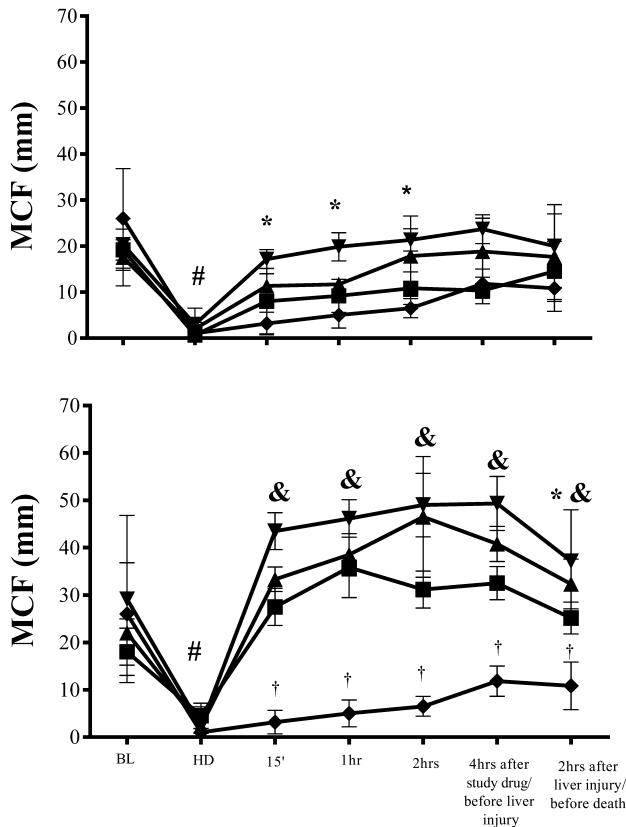
Fig. 3. EXTEM MCF increased in a dose-dependent manner after administration of fibrinogen. Quantities of 300, 450, and 600 mg/kg increased MCF above BL. Top— (■) 37.7 mg/kg; (▲) 75 mg/kg; (▼) 150 mg/kg; (◆) saline. #p < 0.0001 versus BL; *p < 0.05 versus saline for 150 mg/kg. Bottom—(■) 300 mg/kg; (▲) 450 mg/kg; (▼) 600 mg/kg; (◆) saline. #p < 0.0001 versus BL; &p < 0.001 versus saline; *p < 0.05 versus saline for 300 mg/kg; †p < 0.01 versus BL.
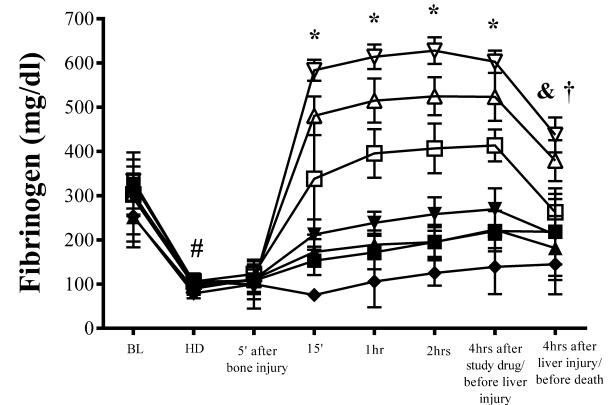

Fig. 4. Administration of fibrinogen concentrate resulted in a dose-dependent increase in plasma fibrinogen concentration. (■) 37.5 mg/kg; (▲) 75 mg/kg; (▼) 150 mg/kg; (□) 300 mg/kg; (△) 450 mg/kg; (▽) 600 mg/kg; (◆) saline. #p < 0.0001 versus BL; *p < 0.001 versus saline for 150, 300, 450, and 600 mg/kg; &p < 0.001 versus saline for 450 and 600 mg/kg; †p < 0.01 versus saline for 300 mg/kg.

p < 0.0001). Administration of fibrinogen did not shorten the prolonged clotting time but increased INTEM MCF (Fig. 2: Group A, 49 ± 2 mm; Group B, 54 ± 5 mm; Group C, 63 ± 4 mm; Group D, 68 ± 2 mm; Group E, 71 ± 3 mm; Group F, 73 ± 3 mm; p < 0.001 vs. saline for dosage Groups C-F 15 min after completion of fibrinogen infusion). Also the alpha angle was increased after fibrinogen administration compared to the saline group (p < 0.05 vs. saline for all dosage groups 15 min after completion of fibrinogen infusion). Treatment with 150 mg/kg fully restored BL MCF values. Higher dosages increased INTEM MCF to a maximum of 80 mm, where a plateau was reached (Fig. 2).

Similarly, EXTEM MCF (Fig. 3) decreased to 2 ± 3 mm after HD compared to 22 ± 9 mm at BL (p < 0.0001). Following the same pattern as described for INTEM MCF, treatment with fibrinogen also increased EXTEM MCF in a dose-dependent manner: Group A, 10 ± 7 mm; Group B, 11 ± 3 mm; Group C, 17 ± 2 mm;

Group D, 28 ± 4 mm; Group E, 33 ± 3 mm; and Group F, 44 ± 4 mm. MCF in the saline group slightly increased to a maximum of 7 ± 2 mm 4 hours after drug infusion; however, it always remained lower than BL.

Plasma fibrinogen concentration

Plasma fibrinogen concentration decreased after HD compared to BL (BL, 295.3 ± 67.2 mg/dL; after HD, 96.8 ± 22.6 mg/dL; p < 0.0001; Fig. 4). Treatment with 150 mg/kg fibrinogen concentrate and higher increased plasma fibrinogen concentrations compared to sham at all time points before liver injury (p < 0.001; Fig. 4). Treatment with 300 mg/kg restored fibrinogen levels to BL. Dosages higher than 300 mg/kg increased plasma fibrinogen concentrations above BL. After liver injury fibrinogen plasma concentrations dropped in all groups; however, animals treated with dosages of 300 mg/kg and higher demonstrated elevated plasma fibrinogen concentrations compared to sham even after liver injury.

Clot firmness and plasma fibrinogen concentration

The relationship of INTEM MCF and plasma fibrinogen concentration is shown in Fig. 5. It is clear that MCF reaches a plateau at plasma fibrinogen concentrations of 350 mg/kg. Further increases of plasma fibrinogen concentrations did not cause additional increase in clot firmness.

Standard coagulation assays

PT increased from 11 ± 1 to 17 ± 2 seconds following HD (p < 0.0001 vs. BL). After treatment no significant changes in PT occurred in all groups at any time point. aPTT increased from 19 ± 3 seconds at BL to 22 ± 3 seconds
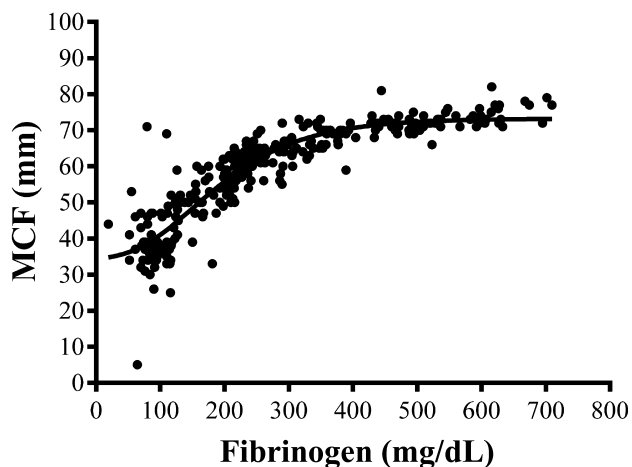
Fig. 5. Relationship between INTEM MCF and plasma fibrinogen concentration: at plasma fibrinogen concentrations of 350 mg/dL MCF reached a plateau. Higher plasma fibrinogen concentrations did not result in further increases in MCF. $R^2 = 0.82$.

(p < 0.0001) after HD. After treatment no significant changes in aPTT occurred in all groups with the exception of Group F, which showed a significantly increased aPTT at all time points after drug administration compared to the other fibrinogen groups and the saline group.

D-dimer was increased compared to the saline group 4 hours after fibrinogen administration in Groups E and F (395 ± 149 and 391 ± 127 ng/mL vs. 181 ± 66 ng/mL; p < 0.01). Groups A, D, E, and F showed an increase in D-dimer at the end of the experiment compared to the saline group (Group A, 399 ± 271 ng/mL, p < 0.05; Group D, 466 ± 131 ng/mL; Group E, 564 ± 209 ng/mL; Group F, 789 ± 425 ng/mL; and saline group, 227 ± 41 ng/mL; p < 0.001). Thrombin-antithrombin and thrombin generation did not differ from the saline group.

PLTs decreased from 281 ± 81 g/L at BL to 74 ± 20 g/L after HD (p < 0.0001). Over the time course of the experiment PLT count recovered to values between 105 and 115 g/L. After liver injury PLT count significantly decreased to 105 ± 16 (Group A), 93 ± 43 (Group B), 122 ± 48 (Group C), 83 ± 16 (Group D), 76 ± 41 (Group E), 76 ± 34 (Group F), and 98 ± 47 g/L (saline group).

Blood count

After HD Hb values decreased from 8.4 ± 0.9 to 3.4 ± 0.4 g/dL (p < 0.0001 vs. BL). Accordingly, Hct decreased from 31 ± 4% at BL to 13 ± 3% after HD (p < 0.0001). After retransfusion of RBCs, Hb and Hct values increased to 5.4 ± 0.5 g/dL and 19 ± 2%, respectively (p < 0.0001 vs. BL for both variables).

Blood loss

Total blood loss after bone and liver injury is shown in Fig. 6. Statistical analysis showed a significant dose–response relationship (p = 0.02), indicating overall decreases in total blood loss with increases in fibrinogen dosages. However, no correlation could be found between blood loss and a specific fibrinogen dose.

Animals receiving 150 mg/kg fibrinogen and higher dosages showed a significant increase in clotting capability as shown by a significant increase in clot weight, formed on the liver surface after the injury (also shown in Fig. 6).

Micro- and macroscopic examinations for thrombosis

Dissection and macroscopic examination of all internal organs did not show any signs for thrombosis or thromboembolic events. Neither did the histologic examination of all tissue slides provide any signs for arterial or venous thrombosis.

Survival

There was no difference in survival among all groups in this study. Survival was 50% in the saline group, 50% at 37.5 mg/kg, 66.6% at 75 mg/kg, 66.6% at 150 mg/kg, 50% at 300 mg/kg, 50% at 450 mg/kg, and 66.6% at 600 mg/kg.

DISCUSSION

Using a pig model for dilutional coagulopathy, we investigated the effect of different fibrinogen dosages on blood loss and clot firmness after liver injury. We found that the administration of fibrinogen is able to reverse dilutional coagulopathy in a dose-dependent manner. Thromboelastometric measurements clearly showed that clot firmness was increased and normalized after administration of fibrinogen. In the INTEM test, treatment with 150 mg/kg fibrinogen fully restored BL MCF values; higher dosages (300, 450, and 600 mg/kg) increased MCF to BL levels and slightly above. All animals showed a dose-dependent increase in plasma fibrinogen concentration, which was stable for the entire time of the experiment. Dosages of 300 mg/kg fully restored BL plasma fibrinogen concentrations. Higher dosages increased plasma fibrinogen concentration above BL levels. Only after liver injury did plasma fibrinogen levels decrease as a result of massive blood loss.

It is very important to note that despite the fact that plasma fibrinogen concentration increased above BL levels, INTEM MCF did not follow this increase in a linear manner: At plasma fibrinogen concentrations of 350 mg/dL MCF reached a plateau; further increases in fibrinogen concentration did not lead to increases in MCF. Although the mechanism behind this finding is not clear at present, these data suggest a possible safety mechanism that prevents the coagulation process from overreacting in a situation of excessive fibrinogen availability, which is a normal or physiologic reaction in patients with severe
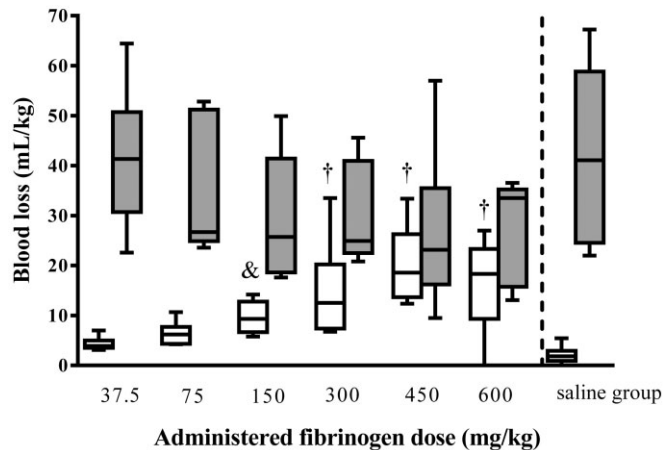
Fig. 6. Blood loss after liver injury. Data are presented as box-and-whisker plot (minimum, first quartile, media, third quartile, maximum). Increasing fibrinogen treatment dosages resulted in increased clot weight (mg/kg, □) and decreased blood loss (mL/kg, ▨). &p < 0.05 versus saline; †p < 0.01 versus saline.

infection or inflammation. Speculating on possible mechanisms, the presence of PLTs seems to be crucial, since the EXTEM data do not suggest such a behavior: One must keep in mind that the EXTEM was performed in plasma and not in whole blood; this change in test performance accounts for the fact that, as seen in previous experiments, swine PLTs cannot be completely blocked by adding of cytochalasin D. The performance of an EXTEM test in plasma, where PLTs are practically absent, can therefore in fact be interpreted as a (modified-) FIBTEM. Especially in the higher dosage groups (300-600 mg/kg) EXTEM MCF increased well above BL. It could be speculated that excessive fibrinogen concentrations inhibit PLT function to prevent hypercoagulation. In fact, even high dosages of fibrinogen did not cause thromboembolic events, which is in accordance with the findings of other studies.[26] Neither macro- nor microscopic signs of thrombotic and/or thromboembolic events were detected. Nevertheless, it must be pointed out that the experiment was terminated 6 hours after fibrinogen administration. Fibrinogen has a half-life of 3 to 4 days, which implies that thromboembolic events could have also happened later. However, this animal model is an acute model and does not allow for longer observation times. Therefore, this question needs to be further investigated with a different experimental approach.

To our knowledge this study is the first study where fibrinogen dosages as high as 12 times the fibrinogen dosage recommended in humans (according to the Austrian Society for Anaesthesiology, Reanimation and Intensive Care Medicine, (http://www.oegari.at/web_files/dateiarchiv/116/Empfehlung Management der Traumablutung 2012.pdf) have been administered in vivo.

It is important to note that in the current study plasma fibrinogen concentrations were measured with

the Clauss method. Studies have shown that the Clauss method overestimates fibrinogen concentrations if colloids have been administered,[27-29] when the clot detection is performed photooptically. The Clauss method is accurate, however, when clot detection is performed mechanically.[28,30] In this study the optical technique has been used, which implies that the reported plasma fibrinogen concentrations are too high.

Since fibrinogen concentrate is not available in all countries, cryoprecipitate might be used as an alternative instead. Besides the different safety profile of cryoprecipitate compared to fibrinogen concentrate, one must further keep in mind that for achieving the same fibrinogen plasma concentrations higher volumes of cryoprecipitate might have to be administered to patients.[31]

For this study a combined bone and liver injury trauma model was used. Whereas bleeding resulting from the bone injury site is mainly venous, bleeding from liver injury is a mixed venous and arterial bleeding. This sequential trauma was used to increase the severity of the animal model. However, it must be noted that the trauma resulting from bone injury was most likely too small to induce trombomodulin-dependent traumatic coagulopathy; nevertheless, bleeding from this injury site could be quite severe (it was stopped by compression if 500 mL was reached) and therefore deteriorated the general condition of the animals.

This study also showed decreased blood loss after fibrinogen administration and a dose-dependent increase in clot weight after liver injury. This finding paralleled the data on increases in clot firmness after fibrinogen treatment, indicating that the ability of clot formation was improved after application of fibrinogen. Whereas in saline animals almost no clot could be found above the injury on the liver surface, animals treated with 150 mg/kg fibrinogen and higher showed significantly heavier blood clots, which completely sealed the wound. On the other hand we did not observe a correlation between blood loss and fibrinogen dose. It is possibly due to the variability of blood loss caused by the bone injury. This could be the reason for the fact that even though overall blood loss was significantly reduced in the treatment groups, no correlation to the administered dose was found.

Despite the beneficial effects of fibrinogen administration on blood loss and clot formation, this study did not show a significant effect on survival. Survival was between 50% to 66% in all groups, including the sham group. Previous studies, conducted in the same animal model[13] have

shown that the combinations of fibrinogen concentrate and prothrombin complex concentrate were not only able to reverse dilutional coagulopathy but also had an impact on survival. All animals treated with 200 mg/kg fibrinogen and 35 IU/kg prothrombin complex concentrate survived after severe liver trauma, whereas 80% of the sham animals died. This finding may indicate that additional clotting factors are needed to improve survival under such extreme conditions.

CONCLUSIONS

In summary, this study showed that 150 mg/kg fibrinogen fully restored clot firmness. Additional increases of the administered fibrinogen dose did not further increase clot firmness. No thrombotic or thromboembolic events were observed with fibrinogen dosages as high as 600 mg/kg. These findings suggest a safety mechanism by which high fibrinogen states can be tolerated without leading to thromboembolic complications. Nevertheless, the half-life of fibrinogen is 3 to 4 days, which is considerably longer than the time animals were observed in the current study. This is a clear limitation of this study. Additional investigations are needed to clarify the long-term effect of fibrinogen concentrate on thromboembolic events.

Even though administration of fibrinogen had no effect on overall survival in this study, reversal of dilutional coagulopathy decreased blood loss, which under clinical circumstances could decrease the number of blood transfusions and therefore minimize patient morbidity, address shortage of blood products, and decrease related costs.

KEY MESSAGES

- Dilutional coagulopathy can be reversed with fibrinogen concentrate. Animals treated with fibrinogen concentrate showed significantly reduced blood loss after liver injury compared to untreated animals.
- Dosages of 150 mg/kg fully restored BL clot firmness. Higher doses did not further increase clot firmness. No signs of hypercoagulability such as thrombotic or thromboembolic events were seen even with extremely high fibrinogen dosages (12 times the dose recommended in humans).
- These findings suggest a safety mechanism by which high fibrinogen states can be tolerated without leading to thrombotic or thromboembolic complications.

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